Molar Mass and Size Measurement of Proteins via Tandem Flow Field Fractionation-Light Scattering

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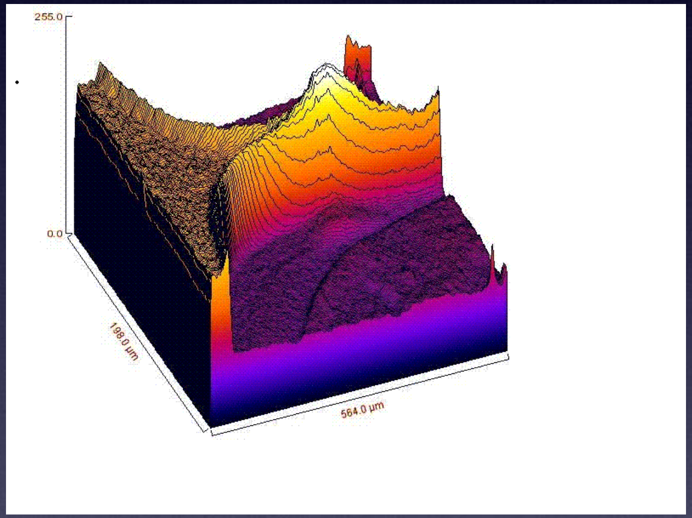
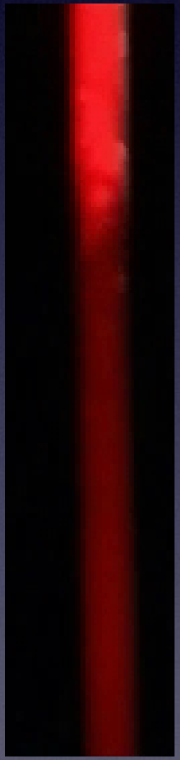
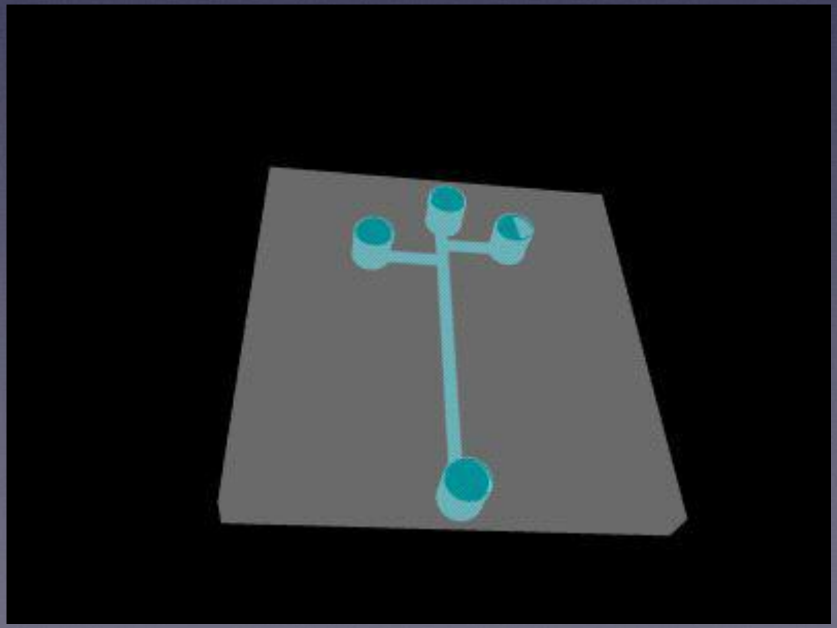
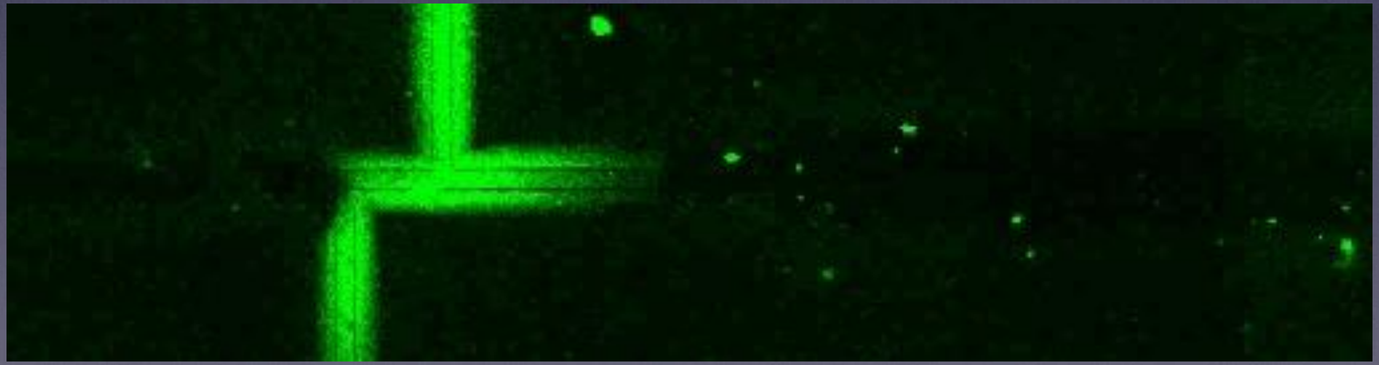

NIST
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PART I
Field Flow Fractionation

The Basics

A Brief History

- J. Calvin Giddings (1930-1996), Prof., Univ. of Utah
  - Described FFF in 1976, Science (1976): 1244-1245
  - Avid outdoors man

The Ground Rules

Particles must be smaller than the length perpendicular to the elution axis

$R_{particle} \ll Thickness_{channel}$

Channel flow must be purely laminar $(Re \ll 1)$

What FFF Can Do?

- Separates particles on the ratio of two (pseudo) orthogonal physicochemical properties
- Can separate “delicate” or “soft” colloidal particles
- Different from chromatography because there is NO STATIONARY PHASE

Typical analytical LC column packing surface area =

~900 m^2

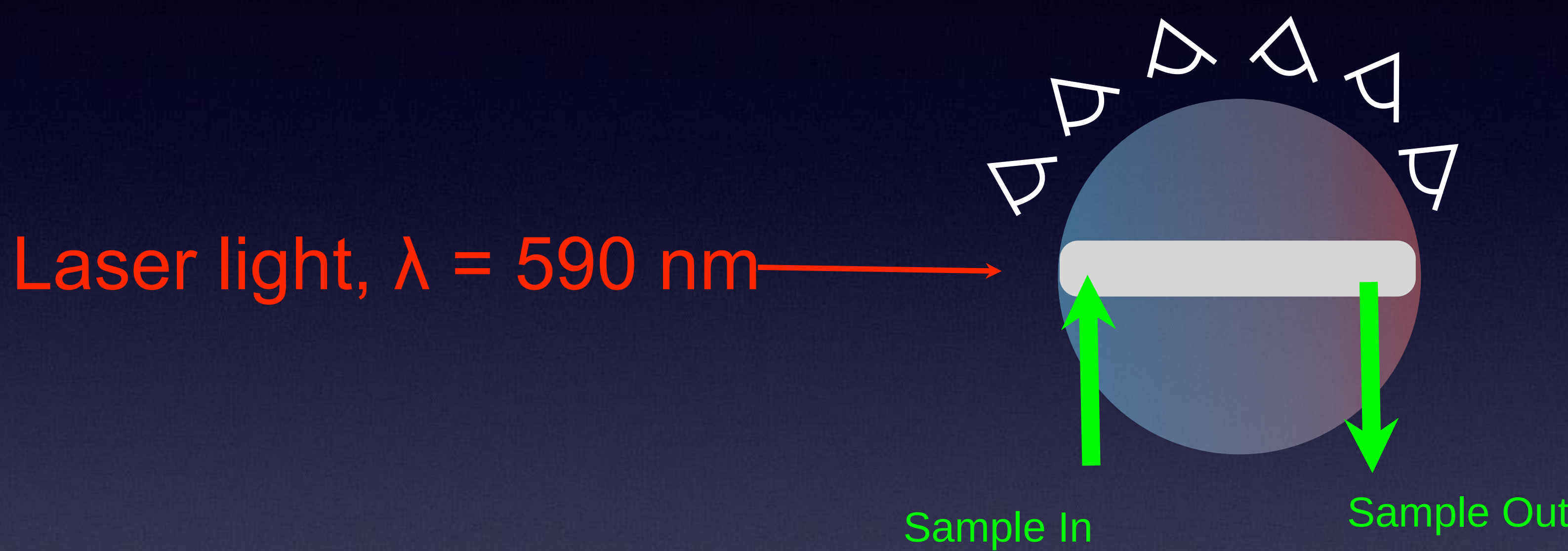
SURFACES

Typical analytical FFF channel surface area =

~0.05 m^2

font sized normalized to a $\log_{10}$ scale of relative surface area

PART II
Absolute Size Measurement



MultiAngle Laser Light Scattering

UV/Vis Spectrometry
Refractive index

$$R(\theta) = K^* M c \, P(\theta)$$

$$K^* = \frac{4\pi^2 n_0^2}{\lambda_0^4 N_A}\left(\frac{dn}{dc}\right)^2 \qquad \frac{1}{P(\theta)} = 1 + \frac{16\pi^2}{3\lambda^2} A_2 \sin^2\frac{\theta}{2}$$

Absolute Size Determination
R_g & D_T or R_h

Radius of Gyration

Measure the *angular dependence* of scattered light

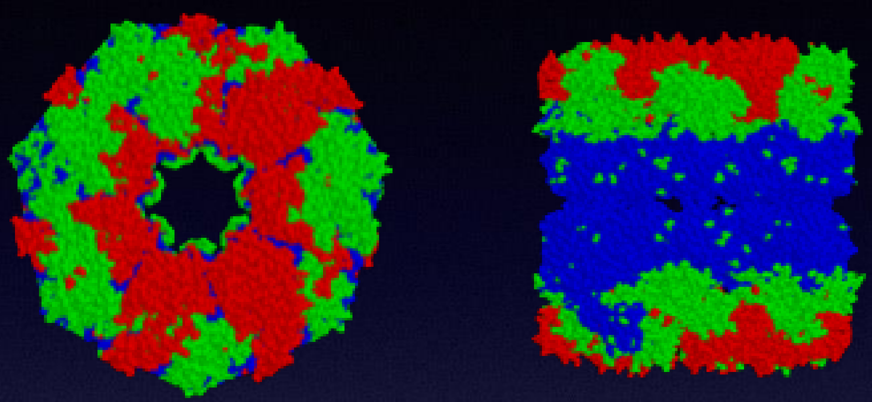
$$R(\theta) = \frac{4\pi^2 n_0^2}{N_A \lambda_0^4}\left(\frac{dn}{dc}\right)^2 MwP(\theta)\left[1 - 2A_2 MwP(\theta)\right]$$

Translational Diffusion Coefficient
-or-
Hydrodynamic Radius

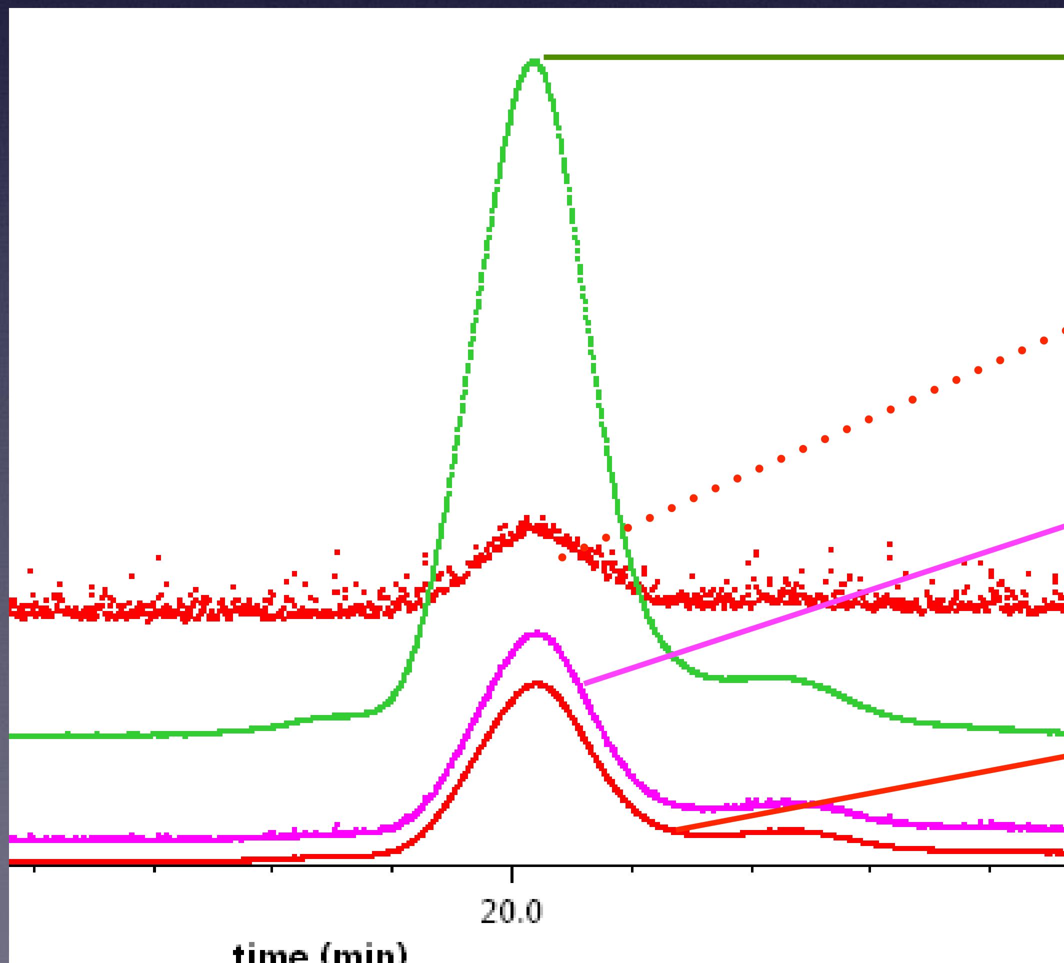
Measure the *time dependent fluctuation* of scattered light

$$g(\tau) = \frac{\langle I(t) \cdot I(t+\tau)\rangle}{\langle I(t)\rangle^2} = e^{-q^2 D_T \tau} \qquad q = \frac{4\pi n_0}{\lambda}\sin(\theta/2)$$

A Case Study: GroEL



Tetradecamer assembly
57 kDa monomer
798 kDa complex
Nickname: “Shake-N-Bake”



dRI (concentration)

low angle LS (noisy)

medium angle LS (high S:N ratio

high angle LS (high S:N ratio)

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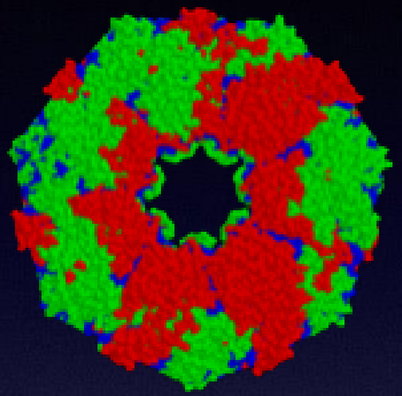

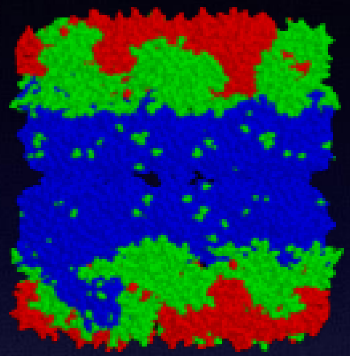

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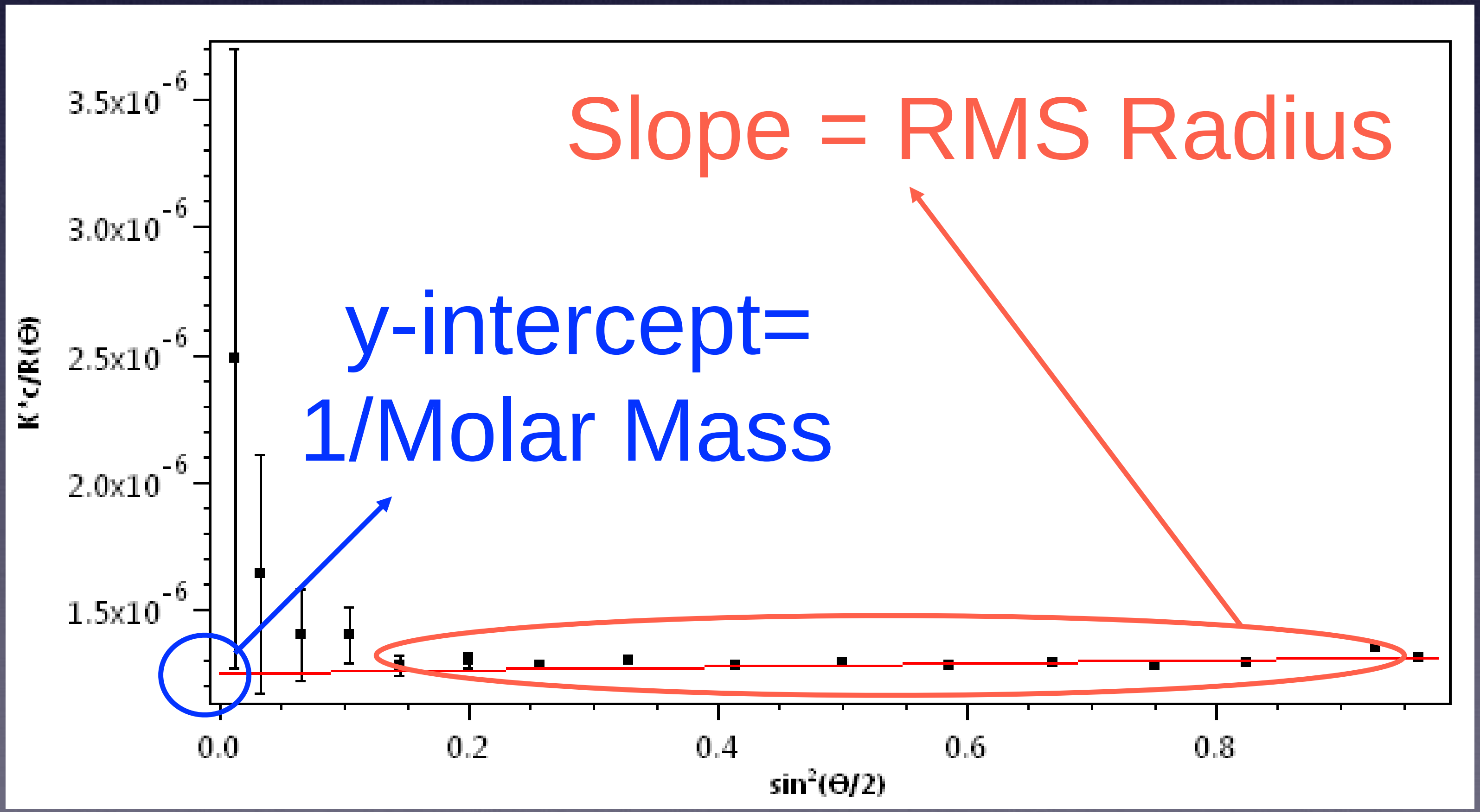

R_n = 22.4 nm
R_w = 22.3 nm
R_z = 22.2 nm
(large uncertainty)

M_n = 775.1 kDa
M_w = 789.6 kDa
M_z = 804.7 kDa
(small uncertainty)

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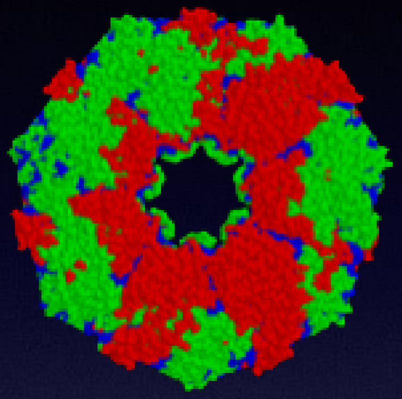

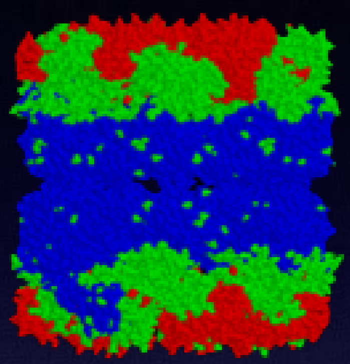

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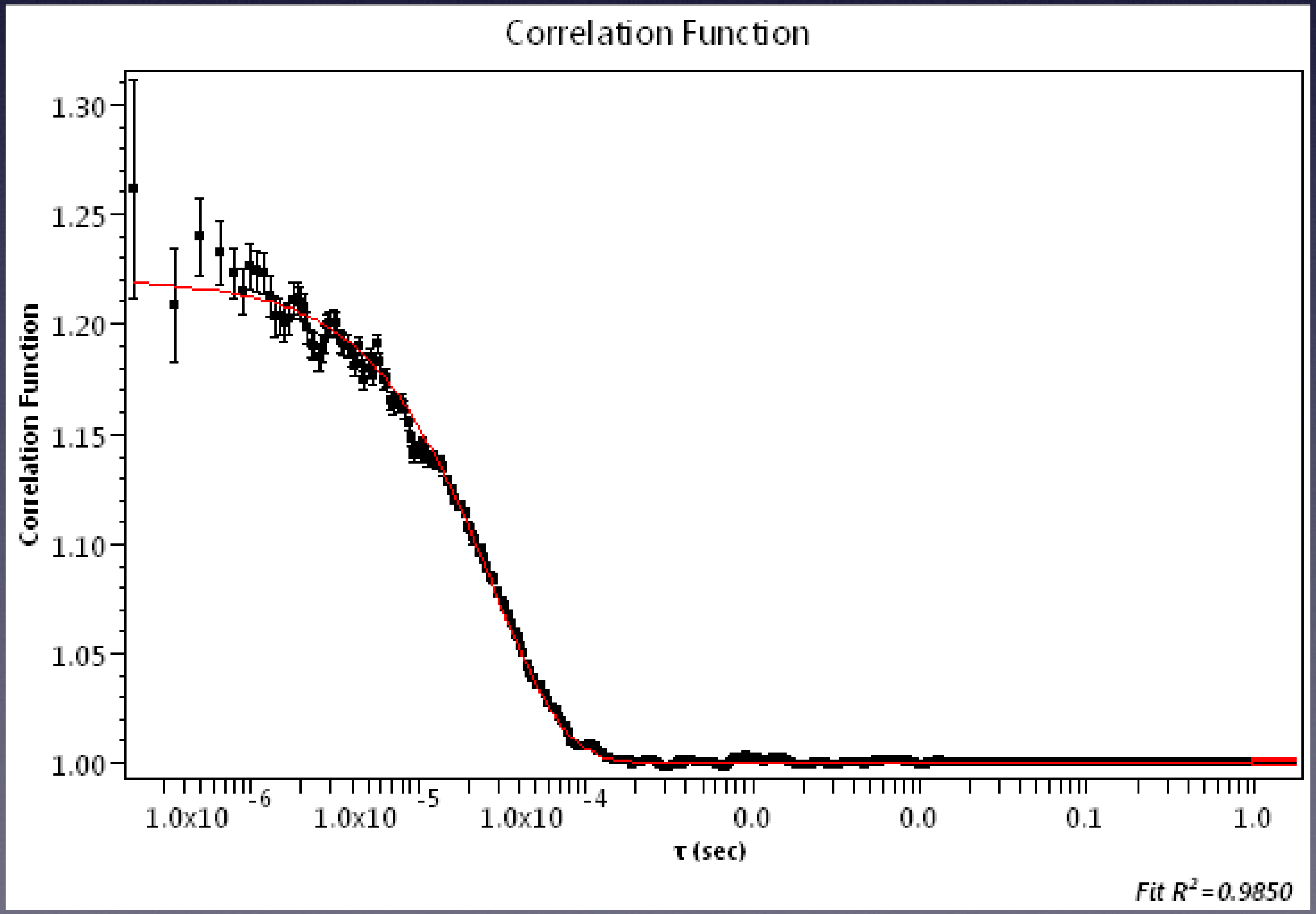

$D_{t\ (avg)} = 4.33e^{-8}\ cm^2/sec$
$R_h = 8.2\ nm$

Future Directions

Inter-comparison of AIST and NIST techniques to these nascent measurement challenges

Exploration of large experimental space:

- Storage conditions
- Lyophilization protocols
- Reconstitution techniques

Move biological measurements to a more quantitative dogma

Ideas

Thoughts

Suggestions